CHLOROPLAST COUPLING FACTOR CF_1 IN SOLUTION

Small-angle X-ray scattering and circular dichroism measurements

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1. Introduction

Recently a proton translocating protein complex was shown to catalyze the photosynthetic ATP synthesis of chloroplasts when the thylakoid membranes are stressed by a transmembrane proton gradient as proposed [1–3]. This complex involves at least the peripheral membrane protein coupling factor CF_1 being the actual ATP-synthetase and a non-identified transmembrane component designated HF_0 [3,4]. From this complex only the coupling factor could be purified to homogeneity. This protein with a molecular weight of about 320 000 [5] could be resolved into five subunit classes ($\alpha, \beta, \gamma, \delta, \epsilon$) by sodium dodecyl sulfate (SDS)–acrylamide gel electrophoresis. They have mol. wt 59 000, 55 000, 37 000, 21 000 and 16 000, respectively [6–8].

The relative staining intensities of the subunit bands from the gels and crosslinking experiments gave evidence for one molecule of CF_1 to be composed of 2α, 2β, 1γ, 1δ and 2ϵ subunits [9–11].

Though conformational changes of CF_1 subunits have been detected during the process of photophosphorylation [12] only little is known on the quaternary structure of the enzyme. Electron microscopic data have shown CF_1 to be spherical in shape, but there exist contradictory results with respect to the diameter of this particle. Previously the diameter of the membrane-bound as well as of the solubilized CF_1 was found to be 150 Å in freeze-etched preparations and 90 Å in negatively-stained material [13]. Thus electron–optical investigations of the quaternary structure of this protein seem not to be reliable. For this reason we studied the structure of the purified CF_1 in solution using the methods of small-angle X-ray scattering and circular dichroism.

The protein was shown to have a radius of gyration of 56.5 Å, a maximal particle diameter of 234 Å, vol. 1 200 000 $Å^3$ and an axial ratio of 1 : 2. The shape of the molecule is best approximated by an oblate ellipsoid of revolution with the axes of $2a$ = 84 Å and $2b$ 168 Å. From the unusually high experimentally-determined volume: dry volume ratio of about 3.4 together with an α-helix content of about 20% it was concluded that channels may exist within the protein molecule.

2. Materials and methods

2.1. *Preparation of the coupling factor CF_1*

CF_1 from chloroplast thylakoid membranes of *Vicia faba* was extracted and purified as in [8]. The enzyme was shown to be pure by gel electrophoresis. It consists of the five subunits $\alpha, \beta, \gamma, \delta$ and ϵ which are present in the protein in a molecular ratio of 2:2:1:1:2 [10]. Before being used for measurements, CF_1 suspended in 2 M ammonium sulfate solution was precipitated by centrifugation and desalted by ultrafiltration dialysis against 50 mM Tris–HCl buffer, pH 7.8. The protein concentrations were determined spectrophotometrically using an extinction coefficient of $\epsilon^{1\%}_{276.5\,nm} = 2.6$.

2.2. *Small-angle X-ray scattering and circular dichroism (CD) measurements*

Small-angle X-ray scattering measurements were done with CuK_α-radiation using a stabilized X-ray generator of the type TUR M62 (VEB Transformatoren- und Röntgenwerk, Dresden) and both an automated small-angle X-ray diffractometer of the Kratky type and a four-slit diffractometer. All measurements were carried out at 20 ± 1°C. Scattering curves were obtained from protein solutions with concentrations of CF_1 of 9.3 g/l, 18 g/l and 112 g/l, respectively, and the corresponding blank curve for the 50 mM Tris–HCl buffer, pH 7.8. Solutions were examined in glass capillaries 1 mm in diameter. For data processing the programme system SAXS was used [14].

CD measurements were carried out with a Roussel-Jouan dichrograph CD 185. The molecular ellipticities were calculated using a mean amino acid residue weight of 114. The protein secondary structure fractions of CF_1 were computed from CD spectra between 210 nm and 240 nm [15] using the reference spectra of [16,17].

3. Results

Guinier plots of the measured scattering curves for CF_1 at various concentrations are shown in fig.1. The radius of gyration, determined from the slope of the scattering curve at zero concentration, was found to be 56.5 Å.

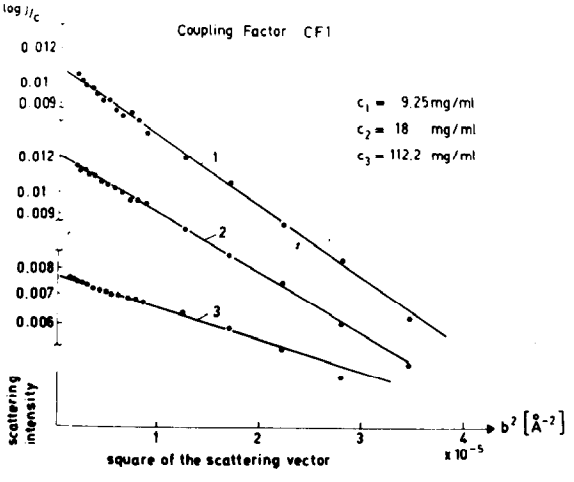


Fig.1. Guinier plot of the scattering curve of coupling factor CF_1 for various concentrations.

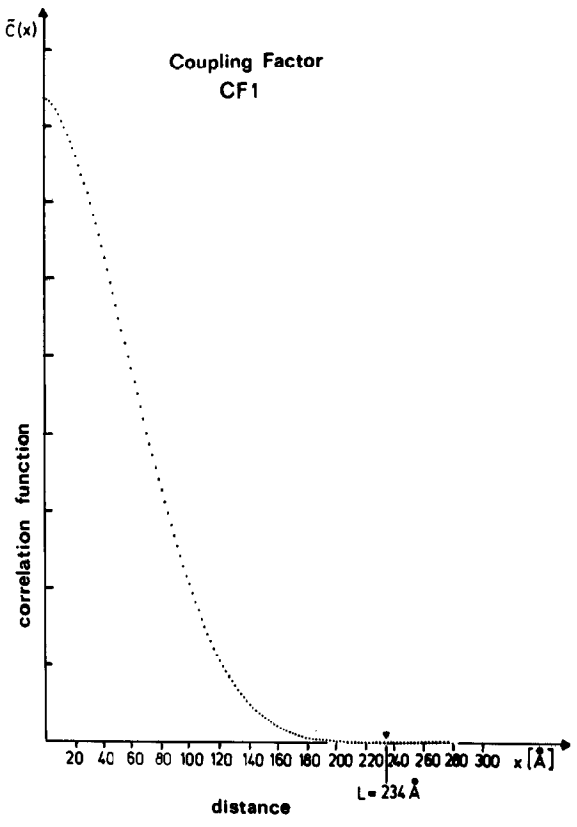


Fig.2. Fourier–cosinus transform of the CF_1 scattering curve.

The slit smeared correlation function $\tilde{C}(x)$ was calculated from the slit smeared scattering curve $j(b)$ by the integral transformation.

$$\tilde{C}(x) = 2\int_0^\infty j(b) \cos 2\pi bx \, db \; [18].$$

The spherically averaged distance distribution obtained by integral transformation of the entire scattering curve [19] yielded a maximum particle diameter of L = 234 Å (fig.2).

The shape of the molecule is determined by comparison of the measured scattering curve of coupling factor with the calculated ones of ellipsoids of revolution. Figure 3 shows that the inner part of the scattering curve of CF_1 is best approximated by an oblate ellipsoid of revolution with the axes of $2a$ = 84 Å and $2b$ = 168 Å. The volume of this model body is V = 1 200 000 Å^3.

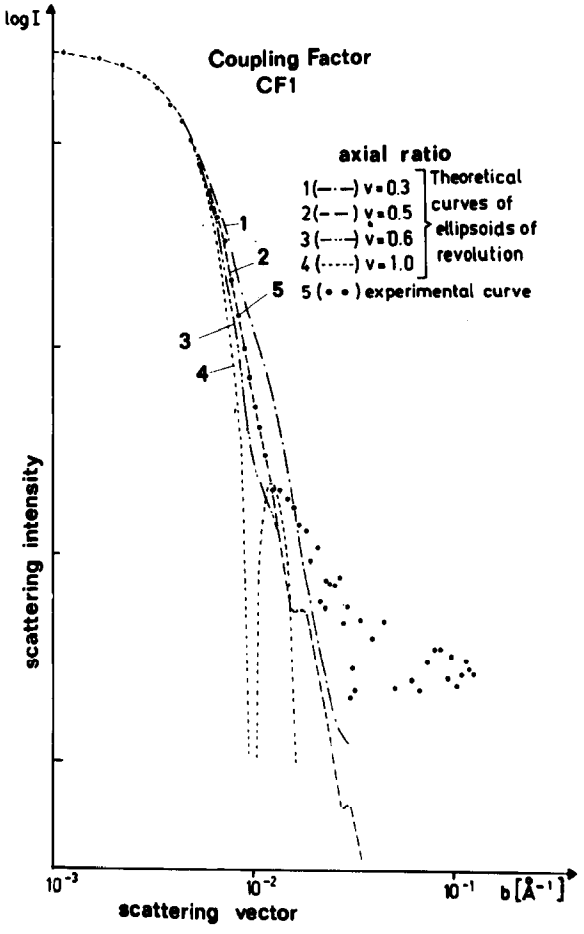
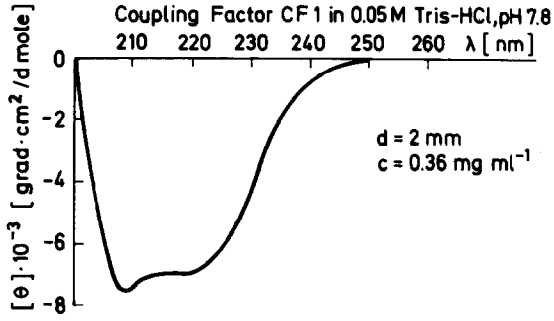


Fig.3. Desmeared CF_1 scattering curve and comparison with the scattering curves of ellipsoids of revolution with different axial ratios. Scattering intensity in arbitrary units.

From the difference between the maximum diameter of this model body (168 Å) and the calculated maximum diameter L = 234 Å together with observed differences in the scattering curves was concluded that the CF_1-molecule is asymmetric in shape.

Figure 4 shows the CD spectrum of the CF_1 and the calculated secondary structure fractions of the protein (α = α-helix, β = β-structure, ρ = unordered structure).

Using the reference spectra [16,17] for calculation of secondary structure fractions 23% α-helices are obtained in both cases whilst the values of β- and ρ-fractions of secondary structures can be considered only as quantitative estimations.



Secondary Structure of Coupling Factor CF1 computed from CD-data (210 nm - 240 nm)

Reference spectra	Percentages of secondary structure		
	α	β	ϱ
Saxena a. Wetlaufer [1971]	23	22	55
Chen Yang a. Chau [1974]	23	14	63

Standard deviations ≈ 1%

Fig.4. CD spectrum of CF_1 and results of the calculation of secondary structure fractions.

4. Discussion

Protein secondary structure fractions of coupling factor CF_1 were calculated from CD spectra. The data indicate nearly 23% amino acids of CF_1-molecule to be in α-helical structures. A similar value was in [9].

The small-angle X-ray scattering data enable some conclusions to be made about the quaternary structure of coupling factor CF_1 in solution.

The linearity of the Guinier plots at different protein concentrations (fig.1) shows that the CF_1 is globular in nature and does not contain partially- or totally-unfolded polypeptide chains. The Guinier plots also indicate the monodispersity of the protein fraction investigated and show no significant dissociation into subunits or intermolecular aggregation of CF_1 molecules.

The unusually high volume of the coupling factor in solution is of particular interest. The volume has been determined from small-angle X-ray scattering data to be $V_{exp.}$ = 1 200 000 Å^3. Using the equation:

$$V_{cal.} = \frac{M \cdot \bar{v}}{N_L}$$

where M is the molecular weight (M = 320 000 g/mol), $\bar{v}$ is the partial specific volume ($\bar{v}$ = 0.737 cm^3/g) and N_L is Loschmidt's number; the dry volume of the CF_1 molecule was calculated to be $V_{cal.}$ = 348 000 $Å^3$. Dividing $V_{exp.}$ by $V_{cal.}$, a volume ratio q of 3.4 was estimated, which is equivalent to a hydration of 1.9 g H_2O/g CF_1. Taking the amino acid composition of CF_1 [8], a maximum hydration of the interphase protein/water of only 0.45 g H_2O/g protein was calculated using the method in [20]. According to our own experience there is a good correspondence between the values of hydration estimated from amino acid composition and the volume ratio q for several soluble enzymes. In the case of coupling factor CF_1, the observed difference between the two values can only be understood if we assume that, besides the structured hydrated surface, channel-like inner cavities exist within the protein molecule. At present, however, it cannot be decided, whether or not these cavities contain water.

Further experiments will be carried out using the method of small-angle X-ray scattering to explain the structure of coupling factor CF_1.

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References

[1] Mitchell, P. (1966) Biol. Rev. 41, 445–502.
[2] Jagendorf, A. T. (1975) in: Bioenergetics of Photosynthesis (Govindjee ed) pp. 413–492, Academic Press, New York.
[3] Hauska, G. A. and Trebst, A. (1977) Curr. Top. Bioenerget. 6, 151–220.
[4] Younis, H. M. and Winget, G. D. (1977) Biochem. Biophys. Res. Commun. 77, 168–174.
[5] Farron, F. (1970) Biochemistry 9, 3823–3828.
[6] Racker, E., Hauska, G. A., Lien, S., Berzborn, R. J. and Nelson, N. (1972) Proc. IInd Int. Cong. Photosynthesis, Stresa (Forti, G., Avron, M. and Melandri, A. eds) pp. 1097–1113.
[7] McEvoy, F. A. and Lynn, W. S. (1973) Arch. Biochem. Biophys. 156, 335–341.
[8] Süss, K.-H. (1976) FEBS Lett. 70, 191–196.
[9] Nelson, N. (1976) Biochim. Biophys. Acta 456, 314–338.
[10] Süss, K.-H. (1976) unpublished experiments.
[11] Baird, B. A. and Hammes, G. G. (1976) J. Biol. Chem. 251, 6953–6962.
[12] Jagendorf, A. T. (1975) Fed. Proc. Fed. Am. Soc. Exp. Biol. 34, 1718–1722.
[13] Garber, M. P. and Steponkus, P. L. (1974) J. Cell Biol. 63, 24–34.
[14] Walter, G., Kranold, R., Gocke, W., Damaschun, G. and Müller, J. J. (1975) Wiss. Zeitsch. Universität Rostock 24, 599–604.
[15] Becker, M., Schälike, W. and Zirwer, D. (1973) Studia Biophys. 35, 203–220.
[16] Saxena, V. P. and Wetlaufer, D. B. (1971) Proc. Natl. Acad. Sci. USA 68, 969–972.
[17] Chen, Y.-H., Yang, I. T. and Chan, K. H. (1974) Biochemistry 13, 3350–3359.
[18] Damaschun, G., Müller, J. J., Samaschun, H., Pürschel, H.-V., Walter, G. and Kranold, R. (1974) Studia Biophys. 47, 63–83.
[19] Damaschun, G., Kley, G., Müller, J. J. and Pürschel, H.-V. (1968) Acta Biol. Med. Germ. 20, 409–412.
[20] Kuntz, I. D. (1971) J. Am. Chem. Soc. 93, 514–516.